Molecular Anatomy, Physiology, and Pathology of Hemoglobin

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All ligated HB= R structure
regardless of heme iron valency or nature of ligand or spin state

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HEMOGLOBIN AND MYOGLOBIN AND THEIR REACTION WITH OXYGEN

Hemoglobin is the protein of the red blood cells that allows vertebrates to transport oxygen from the lungs to the tissues and that helps the return transport of carbon dioxide from the tissues back to the lungs. More may have been written about hemoglobin than about any other molecule. Physicists, crystallographers and chemists of all kinds, zoologists, physiologists and geneticists, pathologists, and hematologists have all contributed to a vast literature. In the erratic ways that scientific research shares with other human endeavours, the multifarious work of that great throng has provided us with an enormous store

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significant. However, the calculation of Matthew et al. were based on wrong coordinates of the histidine in Hb O$_2$.[59s] On correcting that error, they have now found pK$_a$s 5.91 or 6.25 in Hb O$_2$ and 5.86 or 6.00 in Hb CO.[59h] For comparison, Matsukawa et al., using deuterium exchange, found a pK$_a$ of 6.5 in Hb CO in bis-Tris or Tris buffers containing 0.1 M NaCl.[59b] The proton resonance with a pK$_a$ of 7.85 that had been assigned to histidine HC3(146)β[59c] in fact belongs to another histidine, FG4(97)β, which has a raised pK$_a$ because it caps the carboxyl end of an α helix.[161] There is therefore no longer any evidence in favor of histidine HC3(146)β having a high pK$_a$ in Hb CO or Hb O$_2$ in low salt.

Chemical studies have shown consistently that cleavage of the C-terminal histidine or its replacement by another residue or inhibition of its salt bridge in deoxy Hb inhibits a major fraction of the alkaline Bohr effect and that this fraction rises with decreasing concentration of bound anions.[59g] Shih and I recently measured the contribution of His HC3(146)β to the alkaline Bohr effect in saltfree Hb solutions. dpH/dlog Y was −0.2 in Hb A and zero in Hb Cowtown (HC3(146)β Leu), which confirmed that all the chloride-independent part of the alkaline Bohr effect is due to the C-terminal histidines.[591] Their contribution in vivo has never been in dispute.

Chloride ions probably bind to several sites common to the R and T structures, but only those sites are known that are occupied in the T and not in the R structure.[81–83] One such site lies between the α amino group of valine NA1(1)α and the OH of serine H14(131) of the same α chain. The other lies in the central cavity associated with lysines EF6(82)β. When the α amino groups of the α chains are blocked by reaction with cyanate and lysines EF6(82)β are replaced by neutral residues, the effect of chloride on the oxygen affinity becomes negligibly small. The carbamino groups at the amino termini form hydrogen bonds with serines H14(131)α and lysines EF6(82)β.[83–86] Chloride competes with CO$_2$ for binding to valine NA1(1)α, DPG competes with CO$_2$ for binding to valine NA1(1)β, and hydrogen ions compete with CO$_2$ for both these valines.

FERRIC HEMOGLOBIN

The heme irons are subject to auto-oxidation to ferric hemoglobin or methemoglobin, which cannot combine with oxygen. Erythrocytes contain a scavenging enzyme system that reduces methemoglobin to deoxyhemoglobin. That system is composed of cytochrome b$_5$, which I have already mentioned, and an NADH-dependent cytochrome b$_5$ reductase, also known as methemoglobin reductase. In vitro oxy- or deoxyhemoglobin is

oxidized rapidly by ferricyanide or by sodium nitrite. Conversely, methemoglobin can be reduced with dithionite, ascorbate, or borohydride. Methemoglobin is an indicator dye with a pK of 8.1, brown at acid and red at alkaline pH. The color change is due to the ionization of a heme-linked water molecule:

$$Fe^{3+}H_2O \rightleftharpoons Fe^{3+}OH^- + H^+ \left(S = \frac{5}{2}\right) \rightleftharpoons \left(S = \frac{1}{2}\right)$$
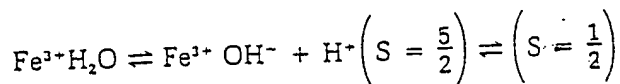
This is accompanied by a transition from high to low spin, i.e., from strong to weak paramagnetism. The water molecule can be replaced by F^-, OCN^-, SCN^-, N_3^-, imidazole or CN^-, where F^- is the weakest and CN^- the strongest ligand. Fluoromethemoglobin is pure high spin ($S = \frac{5}{2}$) and cyanomethemoglobin is almost pure low spin ($S = \frac{1}{2}$), because weak ligands allow the occupation of iron orbitals that point in the bond directions, whereas strong ones restrict occupation to those orbitals that point between the bond directions (Figs. 5–11 and 5–12). Each of these methemoglobin compounds exhibits its characteristic absorption spectrum.[87,162] In cyanide poisoning methemoglobin is used as a scavenger: Intravenous injection of sodium nitrite oxidizes some of the ferrous hemoglobin to methemoglobin, which binds cyanide very firmly, thus protecting the enzyme cytochrome oxidase.

All liganded forms of hemoglobin have the quaternary R structure, regardless of the nature of the ligand and the valency and spin state of the iron.[59] On the other hand, the exact conformation of the heme-ligand complex, and consequently the tertiary structure of the subunits, differs slightly with the nature of the ligand and the spin state of the iron. For example, they are similar in fluoro- and aquomethemoglobin, which are both high spin, and different in cyanomethemoglobin which is low spin.[35] Azidemethemoglobin is also low spin, but the azide ion is longer than the cyanide ion and binds at 120° to the heme axis like oxygen, while in the absence of steric hindrance, cyanide would lie on the heme axis, like carbon monoxide. Hence, the tertiary structure of azidemethemoglobin differs from that of cyanidmethemoglobin. These details modulate the affinity of hemoglobin for different ligands and the equilibrium constant between the T and R structures. For example, at acid pH the allosteric effector inositol hexaphosphate switches the high spin aquo- and fluoromethemoglobins to the T structure, but not the low spin cyanomethemoglobin.

SUBUNIT DISSOCIATION

In dilute solution oxyhemoglobin is partly dissociated into αβ dimers. In 0.1 M NaCl at 20°C the

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dissociation is 3 μM^3. In tion of hem sociation o when meas solutions of taken into a be detected locity of her also be det change of li tetramer-dir hemoglobin of oxyhemog and the mai subunits in t stant into fre measured, t dissociation the rate of e with unlabe been found t sponds to a phosphate, p

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tion constant for the reaction $\alpha_2\beta_2 \rightleftharpoons 2\alpha\beta$ M³. In erythrocytes, in which the concentra- of hemoglobin tetramer is about 4 mM, dissociation of oxyhemoglobin is negligible; but when measuring oxygen equilibrium curves in solutions of less than 60 μM heme, it must be taken into account.[88] Dissociation into dimers can be detected by measuring the sedimentation velocity of hemoglobin in the ultracentrifuge. It can also be detected by light scattering[89] and by a change of light absorption in the ultraviolet. The tetramer-dimer dissociation constant of deoxyhemoglobin is 10^5 to 10^6 times smaller than that of oxyhemoglobin. Owing to the extensive contact and the many hydrogen bonds between the two subunits in the αβ dimers, their dissociation constant into free α and β subunits is too small to be measured, but the first order rate constant of dissociation has recently been determined from the rate of exchange of free ³H-labeled α chains with unlabeled hemoglobin tetramers and has been found to be 4×10^{-3} hours⁻¹, which corresponds to a half time of 173 hours (0.01 M phosphate, pH 7.0, 25°C).[155]

Molecular Dynamics of the Transition of Quaternary Structure

There has been much discussion about this subject, especially over the question whether the salt bridges open and release protons as successive molecules of oxygen or other ligands combine with the T structure, as I suggested,[59] or whether this happens only during the transition from T to R. My suggestion was inspired by kinetic studies that proved the release of protons to be synchronous and linear with uptake of carbon monoxide.[59i, 59k] Recent measurements showed the oxygen affinity at less than 1 per cent oxygen saturation to be strongly pH dependent, which implies that protons are released on combination of the first oxygen when the molecules still remain in the T structure.[59l] This has been confirmed by measurements of the oxygen and pH dependence of the tetramer-dimer equilibrium.[59m] In 0.1 M tris or glycine buffers + 0.1 M NaCl at pH 7.4 and 21.5° C in the absence of DPG, the numbers of protons released per oxygenation step are 0.64 for the first, 1.62 for the second and third combined, and only 0.05 for the fourth. Under these conditions the T to R transition is known to take place mainly on combination of the third oxygen"; since all the protons come from salt bridges, it follows that salt bridges must open and release protons on uptake of oxygen by the T structure as well as on transition from T to R. Few, if any, are released on uptake of oxygen by the R structure. DPG reduces the number of protons released in the first step,[59l] but probably increases the number in the fourth, since a greater

fraction of the molecules would not change from T to R until the fourth step.

FETAL HUMAN HEMOGLOBIN

The transport of oxygen differs in the embryonic, fetal, and adult stages of development. The early embryo obtains oxygen from the maternal interstitial fluid and uses a hemoglobin known as $\zeta_2\epsilon_2$, which has not been crystallized. The developing fetus obtains its oxygen via the placenta, using a hemoglobin known as FII $(\alpha_2\gamma_2)$. This has the same α chain as the adult form, but its β chain, known as γ, differs from the adult form in 39 positions. In fact, there are two γ chain genes that are both expressed and differ only in position H14(136), one coding for Gly and the other for Ala. There is also a minor component, known as hemoglobin FI, in which the valines 1γ are acetylated. In vivo the oxygen affinities of hemoglobins F_{II} and F_I are higher than those of the two adult forms [Hb A $(\alpha_2\beta_2)$ and A2 $(\alpha_2\delta_2)$], which facilitates transfer of oxygen across the placenta from the adult to the fetal circulation.[4, 90] However, when isolated and stripped of phosphates, the oxygen affinities of the fetal forms become lower than those of the adult forms, especially at acid pH.[91, 92] Their higher oxygen affinity in vivo is due entirely to their lower affinity for the allosteric effector, 2,3-diphosphoglycerate (DPG). The alkaline Bohr effect of the fetal forms is almost the same as that of Hb A and is not influenced by the acetylation of Val 1β, but their acid Bohr effect is halved,[90] so that below pH 6 their oxygen affinity rises less than that of hemoglobin A. Deoxyhemoglobin F is more soluble than deoxyhemoglobin A, which has important implications for the pathology of sickle-cell anemia. Hb F is recognized most easily by its higher absorption of light at 290 nm; this is due to the presence of two additional tryptophans per tetramer (tyrosine H8(130)β → tryptophan γ). It can also be recognized by its greater resistance to denaturation by alkali.

The structure of deoxyhemoglobin F was found to be similar to that of deoxyhemoglobin A because most of the 39 amino acid replacements are external and therefore incapable of exercising any influence on the folding of the polypeptide chain; the few internal replacements are accommodated with barely detectable structural changes. The exceptions are the replacements leucine NA3(3)β → phenylalanine γ and glutamate A4(7)β → aspartate γ. The former pushes the amino terminal segment away from the EF corner and from the dyad symmetry axis, while the latter draws helix A closer to helix E and the symmetry axis because residue A4 makes a salt bridge with Lys H10(132)β.[93]

The low affinity of Hb FII for DPG is caused